

## **Variability of Hormonal Stress Markers Collected from a Managed Dolphin Population**

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### **LONG-TERM GOALS**

Quantifying physiological indicators of stress in wild marine mammals and the interrelationships between different stress markers can be used to estimate the impact of anthropogenic stressors on marine mammal populations. The United States Navy, as part of its environmental stewardship, can utilize stress markers to assess the acute and chronic impacts that its actions might have on marine mammals. This approach would permit better mitigation of potential impacts and ensure that Navy activities do not come at a deleterious cost to marine mammal populations.

### **OBJECTIVES**

The objectives of this project are to complete the analyses and publications proposed under a prior award (N000141110436), "Variability of hormonal stress markers collected from a managed dolphin population." Studies conducted under the award N000141110436 produced significant numbers of samples (>10,000), which required more processing time and costs than anticipated at the time the award was received. Similarly, costs for thyroid stimulating hormone (TSH) required to complete the final project of the parent increased above that anticipated at the writing of the original proposal. As a result, slippage in completion of the project occurred as well as a short-fall in funds for materials and labor. The objectives of this effort are to complete the studies and analyses subject to the time slippage and short-fall in funds and to take a step toward completion of a number of publications related to the work of the parent project.

### **APPROACH**

The hormone, TSH (Thyrogen), will be purchased and administered in accordance with the objectives of the parent project. Thyrogen costs ~\$650 per 0.9 g dose and therefore requires a determination of a target dosage that was within budgetary constraints. Based on prior thyroid studies in odontocete cetaceans (St. Aubin 1987; St. Aubin and Geraci 1992; West et al. 2014), it is anticipated that 1.5 g will be required per dolphin to produce a robust thyroid response. A total of 13 doses will be acquired to perform the pilot and main study, which is anticipated to occur in the spring of 2015. Additional funds requested with this proposal ensure that the current cost of TSH will not prohibit the purchase of

hormone processing kits required for remaining analyses identified in the parent project. The TSH challenge will be conducted in accordance with methods described in award N000141110436.

Sample analysis under the parent project will be completed prior to the end of calendar year 2015. In addition, manuscripts associated with the parent project will be submitted for publication. A total of seven publications are anticipated, with the subject of each publication listed below:

1. Seasonal and demographic variation in circulating corticosteroids, thyroid hormones, and catecholamines
2. Diel variation in circulating corticosteroids, thyroid hormones, and catecholamines
3. Effect of chronic elevations in circulating cortisol
4. Impact of megestrol acetate on the hypothalamic-pituitary-adrenal (HPA) axis (*incidental finding*)
5. Effect of ACTH stimulation and an external stressor (stress test) on the acute response of the HPA axis
6. Effect of TSH stimulation on the hypothalamic-pituitary-thyroid (HPT) axis
7. Comparison of HPLC and RIA methods of catecholamine processing

## **WORK COMPLETED**

All TSH stimulations were completed and all hormone analyses for all aspects of the parent project were completed except for the following: aldosterone remains to be processed for the diel variation study; epinephrine and norepinephrine collected during the stress tests remain to be processed. These hormones will be analyzed before completion of calendar year 2015. Additional hormones, the processing of which is supported under a supplemental grant (N-00014-13-1-0770), were also processed in parallel with the remaining hormones (e.g. rT3).

Two publications are in the final stages of preparation and will be submitted within the first month of FY16. The first publication, entitled “Megestrol acetate suppresses the hypothalamic-pituitary-adrenal axis of the bottlenose dolphin (*Tursiops truncatus*),” corresponds to an incidental finding of the suppression of the HPA axis by megestrol acetate, a compound that is used by some marine mammal facilities to control the rut-behavior of male dolphins. This finding was made during the first study of the parent project in which seasonal and demographic influences on stress hormones were investigated. The second publication, entitled “Blubber cortisol reflects circulating cortisol concentrations in bottlenose dolphins,” reports on how chronic elevations in circulating cortisol are reflected in the blubber. Contrary to expectation, the same was not found to be true of feces, although this may be due to how the HPA axis perturbation was achieved.

## **RESULTS**

The use of TSH to stimulate thyroid production met with limited success. The amount of TSH provided was ~67% greater than that used in a prior, recently reported TSH challenge (West et al. 2014). Dosages showed only mild to moderate responses in thyroid hormone release. It is suspected that the result is likely due to the broad variation in TSH across species (as opposed to the conserved

ACTH), which may prove to be an inhibition to achieving true HPT axis stimulation in delphinids. Thus, selection of a commercially available TSH with sufficient power as a secretagogue needs to be identified, or the amino acid sequence of dolphin TSH needs to be determined so that the molecule can be created synthetically.

Results reported in the two prepared manuscripts are found in the report of the parent project (N00014-11-1-0436) and are not repeated here.

## **IMPACT/APPLICATIONS**

The ability to identify stress markers relative to monitoring the health of marine mammal populations is critical to understanding the impact of anthropogenic activities upon those populations. The baseline characterization of stress marker variation in dolphins as a function of seasonality, gender, age, and reproductive status is important to assessing measurements made in wild dolphins. Information on levels and dynamics of stress markers between different matrices provide better estimates of the overall condition of marine mammals sampled in the wild from either blubber biopsies or fecal collections. Sampling from these matrices may be the only means by which handling artifacts can be avoided in cetaceans. Understanding the function and dynamics of the HPA and HPT axes provides fundamental information on the stress response in these marine mammals, which may differ significantly from that of the terrestrial mammals from which most of our understanding is based. The incidental finding of the impact of MegAce on the dolphin endocrine system has broad-scale implications for the welfare of dolphins under human care.

## **RELATED PROJECTS**

Project: Variability of Hormonal Stress Markers Collected from a Managed Dolphin Population (PI Dorian Houser)

This project is the parent project primarily responsible for the performance of the stress hormone characterization. Its goals are the characterization of variability in stress hormones within a population of dolphins as a function of seasonality, time of day, age, gender and reproductive status; the relationship between circulating hormones and those found in blubber and feces; the time course of corticosteroid release and cascade of hormone changes associated with an ACTH stimulation/external stressor; and the time course of thyroid hormone release and cascade of hormone changes associated with a TSH stimulation.

Project: Stress hormones and their regulation in a captive dolphin population (PI Cory Champagne)

This project increases the analysis from the “stress test” and TSH stimulation of the parent project by incorporating metabolomics analyses and measuring the additional compounds of reverse-T3 and corticosteroid binding protein.

## **REFERENCES**

West, K.L., Ramer, J., Brown, J.L., Sweeney, J., Hanahoe, E.M., Reidarson, T., Proudfoot, J. and Bergfelt, D. R. (2014). Thyroid hormone concentrations in relation to age, sex, pregnancy, and perinatal loss in bottlenose dolphins (*Tursiops truncatus*). Gen. Comp. Endocrin. 197: 73-81. doi:10.1016/j.ygcen.2013.11.021

## PUBLICATIONS

- Atkinson, S., Crocker, D., Houser, D., and Mashburn, K. (2015). Stress physiology in marine mammals: how well do they fit the terrestrial model? *Journal of Comparative Physiology B*, 185(5), 463-486. doi:10.1007/s00360-015-0901-0
- Houser, D.S., Champagne, C.D., Crocker, D.E., Kellar, N.M., Cockrem, J., Romano, T., Booth, R.K. and Wasser, S.K. (2015) Natural variation in stress hormones, comparisons across matrices, and impacts resulting from induced stress in the bottlenose dolphin. In: *Effects of Noise on Aquatic Life II*, Popper, A and Hawkins, A, eds. Springer, NY.